Environmental and Experimental Botany, Vol. 29, No. 4, pp. 477–486, 1989
Printed in Great Britain.

0098-8472/89 $3.00 + 0.00
Pergamon Press plc

RESPONSE OF SOLUBLE SUGARS AND STARCH IN FIELD-GROWN COTTON TO OZONE, WATER STRESS, AND THEIR COMBINATION

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(*Received* 25 *April* 1988; *accepted in revised form* 24 *February* 1989)

MILLER J. E., PATTERSON R. P., PURSLEY W. A., HEAGLE A. S. and HECK W. W. *Response of soluble sugars and starch in field-grown cotton to ozone, water stress, and their combination.* ENVIRONMENTAL AND EXPERIMENTAL BOTANY **29,** 477–486, 1989.—Ozone (O_3) stress is known to reduce the growth and yield of a number of crops, and water stress can modify the extent of these effects. Both O_3 and water stress alter the carbohydrate status of plants. Little is known, however, concerning O_3 effects on carbohydrate pools of field-grown plants and whether water stress will modify the carbohydrate response to O_3. Cotton (*Gossypium hirsutum* L. "McNair-235") plants were exposed to five O_3 concentrations in open-top field chambers for 12 hr/day throughout the growing season at two levels of soil water (well-watered or periodically water-stressed). The O_3 concentrations ranged from 0.021 to 0.073 μl/l (seasonal mean 12 hr/day concentration). Plants were sampled from each plot on four occasions encompassing the early- to late-reproductive stages of growth. Soluble sugars (glucose, fructose and sucrose) and starch were measured in leaves, stems and roots at each sampling date. Analysis of variance was performed for main effects and interactions of O_3 and water treatments at each sampling date (O_3 effects were partitioned in linear and quadratic components). Effects of O_3 and water stress on soluble carbohydrates and starch were most common in stems and roots. Ozone suppressed carbohydrate concentrations in all cases where significant O_3 effects were detected in the absence of $O_3 \times$ water interactions. On the other hand, soluble carbohydrate concentrations were greater in water-stressed plant tissues when effects were significant and in the absence of interactions. Water-stress effects on starch were variable. Interactions of O_3 and water stress were not consistent but often included interaction with the quadratic O_3 component.

INTRODUCTION

PLANT productivity is dependent upon the production, translocation, storage, and utilization of carbohydrates.[31] Carbohydrate pools should, therefore, provide relevant information about the response of plants to stresses that reduce productivity. Since ozone (O_3) suppresses photosynthesis and growth of plants,[10,19,25] investigators have assumed that the carbohydrate status of plants would offer a ready explanation for the observed effects of O_3 on growth and yield.[16,19] Suppression of soluble and/or storage carbohydrates has been found in some cases,[3,6,29] whereas in other studies or with other species a stimulation has been found, especially in soluble sugars.[2,5,30] These studies have been done with a number of species and under a variety of conditions, so it is not surprising that no comprehensive explanation for these different results has emerged. Nevertheless, the carbohydrate status of plant tissues is necessarily an important

consideration in understanding the physiological basis of O_3 effects on plant growth and yield.

Carbohydrates also respond to water stress and are considered to be important solutes involved in osmoregulation in some plants.[13,21–23] For example, glucose and starch have been found to accumulate in leaves of drought-adapted cotton (*Gossypium hirsutum* L.).[1] Presumably, glucose acted as an osmoticum and starch reduced cellular osmotic volume, thereby promoting turgor maintenanace at low leaf water potentials. Glucose seemed to be most important in young leaves and starch in older leaves.[1]

Recently, we reported that O_3 suppressed the yield of "McNair-235" cotton when soil moisture was adequate but that the yield response to O_3 was non-significant when soil moisture was limiting.[9] Similar results were found for "Acala SJ-2" cotton when seasonal evapotranspiration rates were high.[28] Although both O_3 and water stress can modify carbohydrate levels in plants, there are no published reports on their interactive effects. The experiment with "McNair-235" cotton[9,20] provided an opportunity to study the effects of O_3 and water stress on carbohydrate pools of field-grown plants, in conjunction with detailed measurements of growth and yield. Our objective was to characterize the influence of O_3 and water stress, singly and in combination, on soluble and storage carbohydrates of field-grown cotton from early- to late-reproductive growth.

MATERIALS AND METHODS

Plant growth

Cotton (*Gossypium hirsutum* L. "McNair-235") was planted with 0.5-m row spacing on 29 May 1985 in a field located approximately 8 km south of Raleigh, NC. The soil consisted of about 30 cm of Norfolk loamy sand (fine-loam, silicious, thermic, Typic Paleudult) overlaying an Appling sandy loam (clayey, kaolinitic, thermic, Typic Hapludult). On 11 June 3.1-m plots (the size needed for open-top chambers) were selected based on stand uniformity and plants were thinned to a final stand of 11 plants/m row. On 18 July, prior to closure of the canopy, rows were removed to give a 1-m row spacing. Further details of the plant culture are given in Heagle *et al.*[9]

Ozone treatments

The experiment consisted of five O_3 levels and two soil moisture levels with two replicates (plots) for each O_3–soil moisture combination for a total of 20 open-top chamber plots. Treatments were randomly assigned to selected plots. The five O_3 treatments were charcoal-filtered air (CF), non-filtered air without added O_3 (NF) and three NF treatments with O_3 added in different proportions of ambient O_3 using dispensing and monitoring methods as described previously.[7,8] The three proportional treatments were approximately 1.2, 1.4 and 1.7 times ambient O_3 (NF × 1.2, NF × 1.4, and NF × 1.7, respectively). The treatments spanned concentrations found in clean to highly O_3-polluted air. Daily 12-hr ozone additions (0800–2000 EST) began on 28 June when plants were approximately 20 cm tall, with an average of four expanded secondary leaves, and continued until 30 October.

Soil moisture treatments and leaf water potential measurements

The two soil moisture treatments were established during periods of low rainfall by differential irrigation of well-watered (WW) and water-stressed (WS) plots. Soil moisture was measured in all plots with a neutron probe (CPN Model 503, Campbell Pacific Nuclear, Pacheco, CA) at a depth of 23 cm. The soil moisture criteria used to determine when irrigation occurred were soil matric potentials of -0.10 and -0.30 MPa for WW and WS plots, respectively. When needed, approximately 2.5 cm of water was applied in each plot with drip tubing located at the base of plants in each row. Leaf water potential of leaf discs from the third or fourth open leaf (counting down from the growing point) was measured between 1100 and 1300 EST during stress cycles with thermocouple psychrometers using chambers similar to those reported by Brown and Collins.[4] Further details of soil and plant water status were reported by Heagle *et al.*[9]

Plant sampling

Five adjacent plants per plot were sampled on 29 July (61 days after planting, DAP), 12 August (75 DAP), and 3 and 23 September (97 and 117 DAP). Two of the five plants sampled were randomly selected for non-structural carbohydrate

analysis. The remaining three plants were used for a study of plant growth.[20] Sampling alternated between the east and west row at each successive date and samples were taken in random order between 1100 and 1500 EST. The intact plants were removed by carefully extracting roots from soil which had been loosened with a spade. Two plants were left as borders between each group of five plants sampled since roots of adjacent plants were disturbed during plant removal.

Immediately after removing each sample from the field, the plants were separated into leaves, stem structures (main stem, branches and petioles), roots and reproductive structures. Tissues from the two plants were then pooled. Because of plant size on the last three sampling dates, tissues were subsampled by quickly cutting the tissues from the pooled sample of two plants into small pieces (leaves into pieces less than 4 cm^2 and stems and roots into segments less than 1 cm long), thoroughly mixing the samples, and then removing a subsample. (The remainder of the plant tissues were oven-dried and weighed to determine total tissue biomass.) The subsampled tissues were immediately frozen in liquid N_2, and placed on dry ice until they could be stored in a freezer at $-10°C$. The tissues were then freeze-dried, weighed, ground, and stored in air-tight vials. Biomass of reproductive structures (squares, flowers and bolls) was measured but they were not analyzed for carbohydrate content.

Carbohydrate analysis

Fifty-milligram samples were extracted three times in ethanol (80% v/v) at 80°C. An aliquot of the supernatant was enzymatically analyzed for sucrose (SUC) and hexoses [glucose (GLU) and fructose (FRU)] by the method of Jones *et al.*[12] as modified by Kerr *et al.*[14] The pellet, containing starch (ST), was enzymatically digested and analyzed for GLU by the same procedure as the soluble sugars. Starch was expressed as GLU equivalents.

Calculations and statistical analysis

Carbohydrate values were expressed as mg carbohydrate per g dry weight of tissue. Total non-structural carbohydrate (TNC) was calculated as the sum of GLU, FRU, SUC and ST. Carbohydrate data from each sampling date were analyzed by analysis of variance for O_3 and water treatment effects and their interactions. Sums of squares for O_3 main effects and $O_3 \times$ water treatment interactions were partitioned into linear, quadratic, and lack-of-fit (lof) components. Partitioning of effects into polynomials of higher order was not included in the reported analyses since this did little to improve biological interpretation of the data. Any significant (by F-test; $P < 0.05$) component was accepted as representative of the O_3 dose–response relationship only if the accompanying test for lof was non-significant. The Statistical Analysis System[26] was used in all analyses.

RESULTS

Ozone doses and soil and leaf water conditions

During the experimental period (28 June–30 October) the ambient O_3 concentration (seasonal mean 12 hr/day, 0800–2000 EST) was 0.044 μl/l. Concentrations in chambered plots (combined for soil moisture treatments and replicates) were 0.021, 0.041, 0.051, 0.061, and 0.073 μl/l for the CF, NF, NF × 1.2, NF × 1.4, and NF × 1.7 treatments, respectively.

The soil matric potential averaged -0.03 MPa in WW plots and -0.13 MPa in WS plots over the 30 occasions that it was measured. Midday leaf water potentials were greater in WW plots than in WS plots each time they were measured. The averages were -1.5 and -2.1 MPa in the WW and WS plots, respectively, on days measurements were made. Leaf water potentials were -2.0 MPa or less on five occasions during the season in WS plots but only once in WS plots. (See Heagle *et al.*[9] for more detail on O_3 doses and soil and plant water status.)

Plant growth and development

At the first sample date (61 DAP), plants had achieved approximately 20–25% of maximum biomass and 40–60% of maximum leaf area attained during the growing season. Production of squares had begun, but no bolls were present on the plants. Reproductive biomass accounted for less than 1% of the total plant biomass at this time. By the last sample date (117 DAP), the plants had achieved maximum total biomass and production of reproductive biomass was at least

90% complete. Effects of the O_3 and water treatments on growth and development are presented in detail in MILLER *et al.*[20]

Tissue concentrations

The concentration of TNC and the individual carbohydrates in all tissues varied to some extent among sample dates (Tables 1, 2 and 3). This is to be expected since the samplings encompassed the early- to late-reproductive stages of development. The concentration of TNC usually reached a maximum at 97 DAP which corresponded to the time that plants had reached maximum vegetative biomass and reproductive production was less than 50% complete.

With leaves, the linear component for O_3 was significant for TNC at 61 DAP and for GLU at 117 DAP, in the absence of significant $O_3 \times$ water treatment interactions (Table 1). (Significance of main effects and interactions was accepted only if accompanying tests for lof were non-significant.) In each case, suppression of carbohydrate concentration with increasing O_3 treatment concentration was indicated. Main effects of water occurred only on 61 and 75 DAP, when $O_3 \times$ water interactions were non-significant. Glucose concentrations were greater in WS plants on both dates, whereas TNC in WS plants was greater on 61 DAP and ST and TNC were less on 75 DAP. A significant $O_3 \times$ water interaction occurred for ST at 75 DAP. On this date, ST concentrations were greater at O_3 concentrations above CF in WW plants, but in WS plants O_3 suppressed ST at the intermediate concentration and elevated ST at the highest O_3 concentration. At 117 DAP, significant $O_3 \times$ water interactions occurred for ST and TNC. In WW plants, both ST and TNC were increasingly suppressed by O_3 concentrations greater than CF, whereas in WS plants both were elevated by O_3 in NF air or in the intermediate O_3 level and suppressed at higher O_3 concentrations.

Effects of O_3 on carbohydrates in stems were more common than in leaves and were most frequent at 61 and 117 DAP (Table 2). In the absence of $O_3 \times$ water interactions, significant O_3 main effects were linear and increases in O_3 concentrations above that in CF air suppressed carbohydrate concentrations. As with O_3 effects, water treatment effects were most common at 61 and 117 DAP. In all cases for which water treatment affected individual carbohydrates or TNC, the concentrations were higher in stems of WS plants than in WW plants. Ozone $\times$ water interactions were significant for GLU, ST and TNC on the last two sample dates. These interactions involved the quadratic component for the O_3 effect and they occurred primarily because intermediate levels of O_3 caused elevated concentrations of GLU, ST and TNC in WS plants but these O_3 levels tended to suppress the concentrations in WW plants.

In roots, in the absence of interactions with water, O_3 effects were linear and generally indicated progressive suppression of carbohydrate concentrations with increasing O_3 concentration (Table 3). When main effects of water treatment were significant and interactions did not occur, water stress usually stimulated carbohydrate concentrations (the only exception was ST at 75 DAP). Eight significant $O_3 \times$ water interactions occurred and six involved the quadratic O_3 component. At 61 DAP, O_3 effects on SUC were slight but the significant interaction reflected different effects of intermediate O_3 concentrations (i.e. a stimulation in WW plants and suppression in WS plants). At 97 DAP, concentrations of FRU were low but a stimulation by intermediate O_3 levels was found in WW plants and effects of O_3 were inconsistent in WS plants. The effects of O_3 on ST concentrations in WW plants at 97 DAP also were inconsistent, whereas ST concentrations were generally suppressed with increasing O_3 in WS plants. At 117 DAP, carbohydrate concentrations in WW plants declined with increasing O_3 except for a slight stimulation at the highest O_3 concentration. WS plants at 117 DAP displayed a general stimulation of carbohydrate concentrations at intermediate O_3 levels and a decline at the highest O_3 concentration.

DISCUSSION

Previous results with various plant species have shown stimulation of,[2,5,30] suppression of,[5,6,29] or no effect[3,11] on soluble carbohydrate and/or ST concentrations due to O_3 stress. In some cases the results differed among tissues analyzed within an individual plant.[3,11,24,30] The present study with field-grown cotton illustrated that soluble carbo-

Table 1. Ozone and water-stress effects on concentrations of soluble sugars and starch in cotton leaves

Water/ozone treatment†	(μl/l)	61 DAP‡ GLU	FRU	SUC	ST	TNC	75 DAP GLU	FRU	SUC	ST	TNC	97 DAP GLU	FRU	SUC	ST	TNC	117 DAP GLU	FRU	SUC	ST	TNC
		(mg/g)					(mg/g)					(mg/g)					(mg/g)				
WW	0.021	14.8	0.0	10.5	9.5	34.8	13.3	0.0	22.7	35.4	71.3	12.4	0.0	20.3	51.6	84.3	7.0	4.3	27.7	82.7	121.7
	0.041	13.5	0.6	2.8	10.4	27.4	10.6	0.0	17.0	48.8	76.3	16.7	0.0	24.4	76.8	117.9	4.3	4.2	35.7	41.5	85.8
	0.051	12.5	0.0	12.1	10.6	35.2	10.2	0.0	29.5	41.7	81.3	9.2	0.0	21.9	62.5	93.6	0.3	6.5	21.8	21.6	50.2
	0.061	9.7	0.3	8.6	8.2	26.9	8.6	0.0	20.0	41.0	69.6	13.0	2.2	16.0	27.0	58.2	0.0	2.7	21.5	13.6	37.7
	0.073	10.3	2.1	4.1	10.0	26.4	13.8	0.0	28.8	39.3	81.9	14.2	0.0	24.7	38.7	77.6	0.0	3.3	22.6	12.8	38.7
	Mean	12.2	0.6	7.6	9.7	30.1	11.3	0.0	23.6	41.2	76.1	13.1	0.4	21.5	51.3	86.3	2.3	4.2	25.9	34.4	66.8
WS	0.021	19.7	0.6	9.6	10.5	40.4	19.6	0.0	24.0	17.7	61.3	13.1	0.0	22.5	61.0	96.5	5.3	5.3	25.5	31.1	67.3
	0.041	26.1	4.2	7.5	9.4	47.2	26.2	0.0	21.7	17.9	65.7	18.8	0.0	23.0	86.7	128.5	6.0	1.9	29.7	43.5	81.2
	0.051	15.6	2.3	4.8	10.3	32.9	12.6	0.0	23.4	7.3	43.3	15.8	0.0	21.8	32.0	69.6	5.6	5.2	30.9	30.3	72.0
	0.061	10.1	0.0	15.4	9.1	34.5	19.8	0.0	24.2	15.4	59.4	18.6	0.0	22.7	29.1	70.5	2.2	5.4	21.5	13.8	43.0
	0.073	14.0	1.9	9.0	7.6	32.4	19.2	0.0	18.5	23.6	61.2	12.3	0.0	23.4	29.5	65.3	2.6	4.7	23.2	14.6	45.1
	Mean	17.1	1.8	9.3	9.4	37.5	19.5	0.0	22.4	16.4	58.2	15.7	0.0	22.7	47.7	86.1	4.4	4.5	26.2	26.7	61.7
Source	df	Significance of effects of ozone, water stress, and their interactions§																			
O (lin)	1	**	NS	NS	NS	*	NS	—	NS	NS	NS	NS	—	NS	**	*	**	NS	NS	**	**
O (quad)	1	NS	NS	NS	NS	NS	NS	—	NS	NS	NS	NS	—	NS	NS	NS	NS	NS	NS	NS	NS
O (lof)	2	**	NS	NS	NS	NS	NS	—	NS	*	NS	*	—	NS	*	*	NS	NS	NS	NS	NS
W	1	**	NS	NS	NS	*	**	—	NS	**	**	NS	—	NS	NS	NS	NS	NS	NS	NS	NS
O (lin) × W	1	*	NS	NS	NS	NS	NS	—	NS	NS	NS	NS	—	NS	NS	NS	NS	NS	NS	**	*
O (quad) × W	1	NS	NS	NS	NS	NS	NS	—	NS	**	NS	NS	—	NS	NS	NS	NS	NS	NS	*	NS
O × W (lof)	2	**	NS	NS	NS	NS	NS	—	NS	NS	NS	NS	—	NS	NS	NS	NS	NS	NS	NS	NS

† Water treatments are well-watered (WW) and water-stressed (WS). Ozone treatments are the mean 12-hr per day concentrations (μl/l) from 28 June to 30 October.

‡ Plants were sampled at 61, 75, 97 and 117 days after planting (DAP) and the tissue analyzed for glucose (GLU), fructose (FRU), sucrose (SUC), and starch (ST). Total non-structural carbohydrate (TNC) is the sum of GLU, FRU, SUC and ST.

§ Results of analysis of variance for ozone (O) and water treatment (W) effects and their interactions. Ozone effects partitioned into linear (lin) and quadratic (quad) components. O (lof) and O × W (lof) are lack-of-fit for main effects of ozone and ozone × water interactions, respectively. * and ** signify F-ratios significant at 0.05 and 0.01, respectively. NS = non-significant.

Table 2. Ozone and water-stress effects on concentrations of soluble sugars and starch in cotton stems

Water/ozone treatment†	(μl/l)	61 DAP‡ GLU	FRU	SUC	ST	TNC	75 DAP GLU	FRU	SUC	ST	TNC	97 DAP GLU	FRU	SUC	ST	TNC	117 DAP GLU	FRU	SUC	ST	TNC
		(mg/g)					(mg/g)					(mg/g)					(mg/g)				
WW	0.021	28.4	10.6	20.8	31.5	91.3	23.5	7.9	49.3	32.8	113.5	13.7	4.7	31.2	69.4	119.0	11.8	4.0	41.1	62.3	119.2
	0.041	24.7	10.7	25.3	20.6	81.3	25.5	8.8	52.3	39.5	126.1	10.6	4.0	32.9	70.1	117.6	8.1	2.7	29.8	34.0	74.6
	0.051	19.9	6.1	25.9	12.9	64.9	22.1	9.0	46.4	34.4	111.8	6.7	0.9	21.1	58.8	87.3	3.8	1.6	26.8	19.3	51.4
	0.061	15.7	4.8	21.1	15.5	57.0	20.5	8.7	46.5	36.4	112.0	8.3	2.3	19.3	36.3	66.2	0.0	0.0	15.7	8.7	24.4
	0.073	12.7	2.6	22.9	7.8	45.9	18.7	6.7	43.1	24.7	93.2	11.9	1.0	32.8	51.7	97.3	0.7	0.0	19.5	8.3	28.5
	Mean	20.3	7.0	23.2	17.7	68.1	22.1	8.2	47.5	33.5	111.3	10.2	2.6	27.5	57.2	97.5	4.9	1.7	26.6	26.5	59.6
WS	0.021	40.8	20.3	33.8	42.8	137.8	25.6	12.5	58.9	24.3	121.2	13.6	3.2	25.2	55.2	97.1	10.4	4.5	48.2	30.1	93.3
	0.041	34.9	18.7	23.3	41.9	118.7	28.4	10.7	47.0	36.4	122.4	16.7	2.9	36.4	89.2	145.1	10.6	3.5	43.0	69.6	126.7
	0.051	28.3	13.6	24.5	28.5	94.9	19.5	9.0	43.2	22.4	94.1	12.0	1.9	27.6	76.0	117.4	9.7	2.4	51.4	47.8	111.3
	0.061	22.9	10.3	29.5	25.2	87.8	25.9	9.7	53.7	29.8	119.1	12.7	2.7	28.3	68.9	112.5	5.3	5.6	22.2	13.9	46.9
	0.073	17.2	10.4	26.8	39.4	93.7	15.0	7.4	42.0	44.7	109.1	10.6	2.6	29.6	52.8	95.6	2.5	0.4	25.6	18.5	47.0
	Mean	28.8	14.7	27.6	35.6	106.6	22.9	9.8	49.0	31.5	113.2	13.1	2.6	29.4	68.4	113.6	7.7	3.3	38.1	36.0	85.0
Source	df	Significance of effects of ozone, water stress, and their interactions§																			
O (lin)	1	**	**	NS	**	**	*	NS	NS	NS	NS	NS	NS	NS	*	*	**	**	**	**	**
O (quad)	1	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	*	NS	NS	NS	NS	NS	NS
O (lof)	2	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	*	NS	NS	NS	*	NS
W	1	**	**	NS	**	**	NS	NS	NS	NS	NS	*	NS	NS	*	*	**	*	*	NS	*
O (lin) × W	1	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	*	NS
O (quad) × W	1	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	*	NS	NS	*	**	*	NS	NS	**	*
O × W (lof)	2	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	*	NS	NS	NS

† Water treatments are well-watered (WW) and water-stressed (WS). Ozone treatments are the mean 12-hr per day concentrations (μl/l) from 28 June to 30 October.

‡ Plants were sampled at 61, 75, 97 and 117 days after planting (DAP) and the tissue analyzed for glucose (GLU), fructose (FRU), sucrose (SUC), and starch (ST). Total non-structural carbohydrate (TNC) is the sum of GLU, FRU, SUC and ST.

§ Results of analysis of variance for ozone (O) and water treatment (W) effects and their interactions. Ozone effects partitioned into linear (lin) and quadratic (quad) components. O (lof) and O × W (lof) are lack-of-fit for main effects of ozone and ozone × water interactions, respectively. * and ** signify *F*-ratios significant at 0.05 and 0.01, respectively. NS = non-significant.

Table 3. Ozone and water-stress effects on concentrations of soluble sugars and starch in cotton roots

Water/ozone treatment†	(μl/l)	61 DAP‡ GLU	FRU	SUC	ST	TNC	75 DAP GLU	FRU	SUC	ST	TNC	97 DAP GLU	FRU	SUC	ST	TNC	117 DAP GLU	FRU	SUC	ST	TNC
				(mg/g)					(mg/g)					(mg/g)					(mg/g)		
WW	0.021	9.7	0.0	59.7	76.4	145.8	3.8	2.6	58.1	121.2	185.6	6.8	1.6	33.0	125.7	167.1	8.7	4.4	63.4	100.6	177.2
	0.041	10.1	0.0	64.0	65.0	139.1	3.4	1.5	68.0	80.6	153.6	8.1	2.9	35.3	151.1	197.4	6.1	1.1	50.5	46.6	104.3
	0.051	9.0	0.0	63.9	36.1	108.9	2.1	2.8	61.9	104.4	171.2	12.2	4.1	23.4	91.2	130.9	4.3	1.4	33.7	42.9	82.2
	0.061	7.3	0.0	58.5	40.5	106.3	2.2	1.8	54.7	73.2	132.0	6.6	2.2	26.6	86.6	122.0	3.6	0.2	18.9	36.2	58.9
	0.073	5.6	0.0	44.6	14.3	64.5	1.5	2.4	50.7	63.1	117.7	6.5	0.1	45.9	147.5	200.0	8.3	3.2	21.2	51.1	83.8
	Mean	8.3	0.0	58.1	46.4	112.9	2.6	2.2	58.7	88.5	152.0	8.0	2.2	32.9	120.4	163.5	6.2	2.1	37.5	55.5	101.3
WS	0.021	8.8	0.0	63.0	99.5	171.3	5.2	4.6	68.6	73.4	151.8	6.0	1.3	35.4	172.3	214.9	7.6	1.0	65.6	93.2	167.3
	0.041	7.4	0.0	53.4	78.8	139.6	5.0	4.7	62.6	64.7	136.9	6.1	0.9	32.8	182.8	222.6	6.8	1.1	57.5	110.7	176.0
	0.051	7.7	0.0	56.4	77.8	141.9	5.3	5.9	59.2	59.5	129.8	6.4	0.6	35.6	109.8	152.4	11.0	2.5	49.5	76.3	139.3
	0.061	8.6	0.0	64.7	63.8	137.0	3.8	4.0	58.3	55.6	121.8	7.1	1.2	36.2	113.2	157.7	7.6	2.4	46.3	51.3	107.6
	0.073	7.7	0.0	61.4	63.4	132.4	3.2	2.4	60.8	66.8	133.2	6.2	0.4	30.4	98.1	135.0	4.8	1.5	34.1	29.3	69.7
	Mean	8.0	0.0	59.8	76.6	144.4	4.5	4.3	61.9	64.0	134.7	6.3	0.9	34.1	135.2	176.5	7.6	1.7	50.6	72.1	132.0
Source	df	Significance of effects of ozone, water stress, and their interactions§																			
O (lin)	1	*	—	NS	**	**	NS	NS	NS	NS	*	NS	NS	NS	**	**	NS	NS	**	**	**
O (quad)	1	NS	—	NS	NS	NS	NS	NS	NS	NS	NS	NS	*	NS	NS	NS	NS	NS	NS	NS	NS
O (lof)	2	NS	—	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	**	**	NS	NS	NS	NS	NS
W	1	NS	—	NS	**	**	NS	*	NS	*	NS	NS	**	NS	NS	NS	NS	NS	**	*	**
O (lin) × W	1	NS	—	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	*	**	NS	NS	NS	NS	NS
O (quad) × W	1	NS	—	*	NS	NS	NS	NS	NS	NS	NS	NS	*	NS	NS	NS	*	*	NS	**	**
O × W (lof)	2	NS	—	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

† Water treatments are well-watered (WW) and water-stressed (WS). Ozone treatments are the mean 12-hr per day concentrations (μl/l) from 28 June to 30 October.

‡ Plants were sampled at 61, 75, 97 and 117 days after planting (DAP) and the tissue analyzed for glucose (GLU), fructose (FRU), sucrose (SUC), and starch (ST). Total non-structural carbohydrate (TNC) is the sum of GLU, FRU, SUC and ST.

§ Results of analysis of variance for ozone (O) and water treatment (W) effects and their interactions. Ozone effects partitioned into linear (lin) and quadratic (quad) components. O (lof) and O × W (lof) are lack-of-fit for main effects of ozone and ozone × water interactions, respectively. * and ** signify *F*-ratios significant at 0.05 and 0.01, respectively. NS = non-significant.

hydrate and ST concentrations were suppressed by O_3 when effects were significant (in the absence of $O_3 \times$ water interactions). The effects were more common in stems and roots than in leaves.

Possible reasons for O_3-induced suppression of carbohydrate pools, which are well documented, include effects on light and dark reactions of photosynthesis[27] and degradation of chlorophyll[15] which are reflected in lower rates of net CO_2 uptake by plant leaves.[25] Carbohydrate synthesis, ST metabolism, and carbon metabolism in general are affected by O_3.[16] With other diseases or stresses, it has been proposed that a greater amount of assimilated carbon is used to repair stress-induced damage which may increase dark respiration.[18] Stimulation of dark respiration due to O_3 stress is well documented[19] and, thus, could contribute to suppression of carbohydrate levels. Ozone also has been shown to decrease export of recently assimilated carbon from source leaves to sinks[17] which could alter carbohydrate pools.

In the absence of $O_3 \times$ water interactions, water stress stimulated soluble carbohydrate concentrations, whereas the response of ST was more variable. Carbohydrates are one of several solutes involved in osmoregulation, an adaptation that enables the plant to withstand more severe water deficits without wilting.[21] In the present study GLU was elevated in leaves of water-stressed plants at the first two sample dates. Similar stimulation of GLU levels of young leaves has been found in cotton adapted to water stress, although higher ST concentrations were found in older leaves of adapted plants.[1] In our study, ST in leaves was not affected in the absence of an $O_3 \times$ water interaction. Since leaves were not separated according to age in the present study, differences in young and older leaves were not discernible. Significant effects of water stress occurred most frequently in stems in our study and both soluble carbohydrates and ST sometimes were stimulated. Osmotic adjustment to water stress also has been found for roots of cotton.[23] Response of root carbohydrates (mainly ST) to water stress in our study was not consistent across sampling dates, so it is not clear if the changes were related to osmotic adjustment.

Interactions of O_3 and water stress on carbohydrate concentrations were most prevalent at the last two sample dates (97 and 117 DAP) and usually involved the quadratic component for O_3. The nature of the interactions was not completely consistent across tissues or carbohydrates. The most common pattern, however, was a linear suppression of carbohydrate concentrations with increasing O_3 concentrations in WW plants, as contrasted to a stimulation of carbohydrate concentrations at intermediate O_3 concentrations in WS plants. Reasons for this difference in response to O_3 in WW and WS plants are not certain and this finding requires additional research to resolve.

It was not possible to control the O_3 and water stress events according to a strict schedule in this experiment. Thus, the stress conditions were not necessarily the same on and immediately preceding the days on which the plants were sampled. This should be considered when evaluating analyses of carbohydrate pools at specific times. For example, cotton leaves and roots may lose as much as 50% of their osmotic adjustment within 3 days after relief of water stress.[23] In the present study, midday leaf water potentials were about 0.1–0.3 MPa lower in the WS plants than in the WW plants at 61, 97 and 117 DAP. However, at 75 DAP the WS plants were severely stressed with midday leaf water potentials of -1.5 and -2.7 MPa in the WW and WS plots, respectively. This may have resulted in the substantial suppression of ST in leaves and roots and the increase in GLU in leaves in the WS plots at 75 DAP. At other sampling dates, water stress usually stimulated carbohydrate concentrations when significant effects were found.

The periodicity of O_3 stress also should be considered. Dry periods and O_3 episodes are sometimes correlated. Ambient O_3 concentrations were fairly high when WS plants were severely stressed at 75 DAP (0.075 μl/l, 12 hr/day average, compared to ambient O_3 concentrations of 0.045 μl/l or less on the other sampling days). Despite the high O_3 levels at 75 DAP, the effects of O_3 were not especially severe, with only GLU in stems and TNC in roots being significantly suppressed. The water stress experienced before and during this time probably reduced stomatal aperture and thus O_3 uptake, minimizing the O_3 effects. Even the WW plants were somewhat stressed due to the dry, hot conditions, which may

have also minimized O_3 effects in these plants. Furthermore, under chronic O_3 stress, effects of O_3 are usually cumulative. Thus, the carbohydrate concentrations would reflect the total history of O_3 exposure and not only the occurrence of individual episodes.

In general, the effects of O_3 stress on carbohydrate concentrations of the cotton tissues were consistent with effects on growth and yield.[9,20] When significant effects were detected, O_3 usually suppressed carbohydrate concentrations to a greater extent in WW than in WS plants. This pattern is similar to the modification by water stress of the growth and yield responses to O_3. The O_3 effects on soluble carbohydrates and ST were usually the greatest at 117 DAP, which is the period when O_3-accelerated senescence of the plants was becoming quite apparent. Effects of O_3 were quite apparent, however, at 61 DAP, which corresponds to the period when growth effects were first observed.[23]

SUMMARY

Effects of O_3, water stress, and their combination on soluble carbohydrates and ST of field-grown cotton were variable but several generalities were apparent from the data. In all cases where significant O_3 effects were detected and $O_3 \times$ water interactions were non-significant, O_3 caused a suppression of carbohydrate concentrations. The effects of O_3 were most common in stems and roots. Water stress caused a stimulation of soluble carbohydrate concentrations in the absence of interactions. Interactions of O_3 and water stress were not consistent but often included interaction with the quadratic O_3 component.

Acknowledgments—The technical assistance of Emanuel Johnson in processing the plant tissues and performing the carbohydrate analyses is greatly appreciated. We thank David Guthrie for advice and assistance in planting, Susan Corda for technical assistance, Robert Philbeck and Van Poole for systems maintenance and calibration, and Virginia Lesser for statistical advice. We appreciate the help of Emory York in freeze-drying the plant tissues.

Cooperative investigations of the USDA-ARS and the North Carolina State University. Paper No. 11575 of the Journal Series of the North Carolina Agricultural Res. Serv., Raleigh, NC 27695-7601. Research partly supported by an Interagency Agreement between the Environmental Protection Agency and the USDA; Interagency agreement number AD-12-F-1-490-2. The use of trade names in this publication does not imply endorsement by the North Carolina Agricultural Research Service or the USDA of the products named, nor criticism of similar ones not mentioned. Although the research described in this article was partly funded by the US EPA, it has not been subjected to the EPA's paper and policy review and may not reflect the views of the Agency.

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